



Modulation of Pain with Transcranial Direct Current Stimulation and Diffuse Noxious Inhibitory Controls

Citation

Reidler, Jay S. 2014. Modulation of Pain with Transcranial Direct Current Stimulation and Diffuse Noxious Inhibitory Controls. Doctoral dissertation, Harvard Medical School.

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Acknowledgements

I feel deeply grateful to the many people who helped shaped my research career over the past 5 years. Since my first year in medical school, Dr. Felipe Fregni has been an outstanding mentor. He has taught me so much about research, medicine, and life generally. His commitment to discovery and innovation in medicine has been a true inspiration and I look forward to carrying forward the many lessons I've learned from him throughout my career. Dr. Fregni provided exceptional support and advice as I planned and completed this study, carefully reviewed the manuscript, and offered countless helpful suggestions.

I would like to acknowledge and thank the other lab members and collaborators who contributed to this study: Mariana Mendonca randomized the participants in this double-blinded experiment and administered the active and sham stimulation. Andrew Ellison and Yansong Zhao at Boston University School of Medicine Center for Biomedical Imaging helped with the technical aspects of magnetic resonance spectroscopy scanning of subjects. Drs. Robert Lenkinski and Xiaoen Wang from the department of Radiology at Beth Israel Deaconess Medical Center processed our magnetic resonance spectroscopy data and contributed to the related methods section in the manuscript. Drs. Serge Marchand and Andrea Motta from the University of Sherbrooke helped review and refine the manuscript. Marcus Santana, Lydia Latif, Joao Amadera, Rasheda El-Nazer, Laura Sherman, Lauren Richardson, and Jennifer Schadler provided extremely helpful suggestions and administrative support, without which this study would not have been possible.

Thank you to the other members of our lab with whom I've developed close friendships over the years, including Marcel Simis, Soroush Zaghi, Daniel Pimentel, Joao Amadera, Kayleen Weaver, and Sarah Daly.

Special thanks to Drs. D'Amico, Shields, and Fazio and to Csilla Kiss for their unwavering support since my first day in medical school.

Finally, I want to express my deepest gratitude to my parents, grandparents, siblings, daughter Layla, and wife Estie, who have been immeasurably devoted to me and made me who I am.

Funding

This work was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health, grant number R21 DK081773. The Office of Enrichment Programs at Harvard Medical School and the Laboratory of Neuromodulation provided me with a stipend during the summer following my first year in medical school, which was spent full-time working on this project. The investigators do not have any conflicts of interest.

Preface

I have been involved in Dr. Fregni's Laboratory of Neuromodulation at Spaulding Rehabilitation Hospital since early in my first year in medical school. During my time in the lab, I have led and contributed to a number of research projects that have resulted in the following works:

1. **Reidler, J.S.**, Nascimento, B., Wu, D.S.K., Carvas, M., Massuyama, B.K., de Oliveira, B.M.R., Zaghi, S., de Rezende, D.T., El-Nazer, R., Walyson, N.G., Merabet, L., & Fregni, F. (2010). Modulation of inhibitory systems to enhance motor rehabilitation: insights for the use of noninvasive brain stimulation. *Psychology & Neuroscience*, 3 (2), 151-60.
2. **Reidler, J.S.**, Zaghi, S., & Fregni, F. (2011). Neurophysiological Effects of Transcranial Direct Current Stimulation. In *Neurofeedback and Neuromodulation Techniques and Applications*. London: Elsevier Inc., eds. Coben and Evans, pp. 319-50.
3. **Reidler, J.S.**, Mendonca, M.E., Santana, M.B., Wang, X., Lenkinski, R., Motta, A.F., Marchand, S., Latif, L., & Fregni, F. (2012). Effects of Motor Cortex Modulation and Descending Inhibitory Systems on Pain Thresholds in Healthy Subjects. *Journal of Pain*, (13)5, 450-8.
4. Simis, M., **Reidler, J.S.**, Macea, D.D., Duarte, I.M., Wang, X., Lenkinski, R., Petrozza, J.C. & Fregni, F. (under review). Investigation of Central Nervous System Dysfunction in Chronic Pelvic Pain Using Magnetic Resonance Spectroscopy and Non-Invasive Brain Stimulation.

This thesis focuses on an investigation that I led under Dr. Fregni's supervision over the course of my first three years in medical school, which was published in the *Journal of Pain* (Reidler et al., 2012; #3 above). The introduction and discussion sections of this thesis also draw on some of the work from my other publications. I worked on the present investigation full-time during the summer between my first and second year in medical school and part-time during the semesters leading up to and following that summer. I was involved in all aspects of the experiment including study design, participant recruitment, questionnaire administration, sensory testing, magnetic resonance spectroscopy scanning, statistical analyses, manuscript preparation, submission for peer review, and critical revision. Many other people contributed to this study and I have outlined their contributions in the Acknowledgments section above.

Of note, the present study in healthy subjects was designed in conjunction with another investigation, which examines the use of tDCS for treating chronic pelvic pain. While the present study independently examines the pain modulatory effects of tDCS and DNIC in healthy subjects, we eventually compared our findings in this control group to those observed in patients with chronic pelvic pain. The chronic pelvic pain study was recently completed and I am second author on the manuscript that has been submitted for publication (Simis et al., *under review*; #4 above). I have chosen to present in this thesis only data for which I was the lead investigator and therefore did not include the chronic pelvic pain study.

Glossary of Abbreviations

ACC: anterior cingulate cortex

BDI: Beck depression inventory

CIPA: congenital insensitivity to pain with anhidrosis

CNS: central nervous system

Cr: creatine

CS: conditioning stimulus

DC: direct current

DNIC: diffuse noxious inhibitory controls

DRG: dorsal root ganglion

EEG: electroencephalogram

GABA: gamma-aminobutyric acid

Gln: glutamine

Glu: glutamate

GM-CSF: granulocyte-macrophage colony stimulation factor

¹H-MRS: proton magnetic resonance spectroscopy

LTP: long-term potentiation

M1: primary motor cortex

MAPK: mitogen-activated protein kinase

MEP: motor-evoked potential

mI: myoinositol

MRI: magnetic resonance imaging

MRS: magnetic resonance spectroscopy

NAA: N-acetylaspartate

NAAG: N-acetylaspartateglutamate

NGF: nerve growth factor

NIH: National Institutes of Health

PAG: periaqueductal gray

PLC: phospholipase C

PNS: peripheral nervous system

PPT: pressure-pain threshold

PRESS: point resolved spectroscopy

rTMS: repetitive transcranial magnetic stimulation

RVM: rostroventral medulla

SRD: subnucleus reticularis dorsalis

tDCS: transcranial direct current stimulation

TE: echo time

TENS: transcutaneous electrical nerve stimulation

TMS: transcranial magnetic stimulation

Total NAA: N-acetylaspartate + N-acetylaspartateglutamate

TR: repetition time

TRP: transient receptor potential

TS: testing stimulus

VAS: visual analog scale

VFH: Von Frey hair assessment

Abstract

Background: While pain is essential for physiological functioning, chronic or pathologic pain is responsible for a major burden of disease in society. Novel approaches to treating acute and chronic pain have employed neuromodulatory tools to target the central and peripheral neural structures that mediate pain. Transcranial direct current stimulation (tDCS), for example, is a safe, non-invasive brain stimulation technique that has been shown in preliminary studies to reduce chronic pain when applied to the primary motor cortex. In contrast to this exogenous neuromodulatory approach, diffuse noxious inhibitory controls (DNIC) refers to endogenous pain regulatory mechanisms that decrease pain following introduction of heterotopic noxious stimuli. This thesis explores whether combining these exogenous and endogenous pain modulation approaches synergistically increases the threshold at which pain is perceived.

Methods: We conducted a double-blinded, randomized, placebo-controlled trial with a crossover design to investigate the effects of tDCS and DNIC on pain thresholds in 15 healthy human subjects. Pain thresholds were assessed prior to and following administration of active tDCS, sham tDCS, cold-water-induced DNIC, and combined active tDCS and DNIC. Using magnetic resonance spectroscopy, we examined whether baseline concentrations of brain metabolites such as N-acetylaspartate in pain-related regions of interest were associated with responses to the varying neuromodulatory conditions.

Results: Pain thresholds significantly increased following both active tDCS and the DNIC paradigm. These modulatory approaches appeared to have additive effects when combined. Pain threshold increases after active tDCS were positively correlated with baseline levels of N-acetylaspartate, a marker of good neural function, in the anterior cingulate cortex and negatively correlated with baseline levels of glutamine in the thalamus.

Conclusions: Combining endogenous pain regulatory mechanisms with exogenous stimulation of the motor cortex can more effectively increase pain thresholds in healthy humans. Future studies should examine whether existing pain therapies may be enhanced with noninvasive brain stimulation and activation of DNIC. They should also assess whether brain metabolite levels can be utilized to predict clinical response to therapeutic interventions.

Introduction

Pain represents a major challenge in clinical medicine. It is associated with a wide array of injuries and pathologies, sometimes resulting secondary to discrete causes such as malignancy or surgical intervention, and other times constituting the primary pathology, as in neuropathic pain and fibromyalgia. This thesis explores the possibility of modulating the experience of pain using a non-invasive brain stimulation technique known as transcranial direct current stimulation (tDCS) and endogenous pain regulatory mechanisms known as diffuse noxious inhibitory controls (DNIC). In this introduction, I present an up-to-date review of the literature surrounding the present field of investigation. This includes discussion of the burden of disease associated with chronic pain, the neurobiology of physiologic pain, the peripheral and central sensitization processes involved in the development of pathologic pain, and current limitations in developing new pharmacologic treatments for pain. I then provide background on the two pain modulation approaches examined here, tDCS and DNIC, including their theorized mechanisms of action and neurophysiology. Finally, I detail the aims of the present investigation and our related hypotheses.

Chronic Pain: Burden of Disease

Chronic pain, or pain persisting past three months, is associated with a great burden of disease in society. It is estimated that 1.5 billion people around the world suffer from various forms of chronic pain, including approximately 100 million in the United States alone.¹ This amounts to a higher prevalence than the number of individuals affected by cancer, diabetes, and heart disease combined.^{1,2} Chronic pain is a heterogeneous condition that presents in many forms, such as low back pain, chronic migraine, post-traumatic pain, and chronic pelvic pain. In 2011, the Institute of Medicine estimated that chronic pain generates up to \$635 billion annually

in direct health care spending and lost worker productivity in the United States (about \$2000 annually per capita).¹ Thus, chronic pain represents a major public health challenge requiring innovative treatment approaches.

Physiologic Pain: Function and Neurophysiology

In recent years major advances have been made in our understanding of both physiologic and pathologic pain. Pain is an evolutionarily conserved phenomenon that plays an important protective role in normal functioning.³ Evidence of this can be drawn from rare cases in which genetic mutations lead to a congenital inability to experience pain. For instance, in congenital insensitivity to pain with anhidrosis (CIPA), a genetic mutation involved in nerve development leads to insensitivity to painful stimuli, while still allowing sensitivity to touch and pressure. Individuals with this condition experience recurrent joint dislocations, thermal injuries, unrecognized chronic fractures, auto-amputation of fingers and toes, and ulcerations of the lip, tongue, oral mucosa, and fingertips due to biting.⁴⁻⁶ Physiologic pain signals to an organism when harm, either external or self-inflicted, may occur, and thus appears essential for survival.

Pain is mediated by neural activity that is distributed throughout the peripheral and central nervous system (PNS and CNS, respectively). The peripheral neurons involved in pain sensation are called nociceptors. These are classified by axon type into A-delta ($A\delta$) fibers, which are thinly myelinated and fast-conducting, and C fibers, which are unmyelinated and slow-conducting.^{3,7} This is in contrast to A-beta ($A\beta$) fibers and proprioceptors, which are responsible for non-nociceptive sensory functions such as light touch, movement, and vibration. Nociceptor nerve endings contain a diverse array of ion channels that are responsible for transducing specific noxious stimuli. For instance, hot temperatures are transduced by transient receptor potential (TRP) channels such as TRPV1 and TRPV2, while cold temperatures are

transduced by TRPM8 and Nav1.8.⁸⁻¹⁰ Acid-sensing channels known as ASICs respond to high concentration of protons, while Piezo1 and Piezo2 transduce mechanical pain.^{8,11} When activated, these ion channels stimulate a transient potential that is amplified by sodium channels Nav1.7, Nav1.8, and Nav1.9 in the terminal nerve.^{12,13} When the nerve reaches threshold, an action potential is triggered that transmits the nociceptive information from the peripheral nervous system into the central nervous system. Interestingly, loss-of-function mutations in the Nav1.7 gene (SCN9A) have been reported to cause pain insensitivity, while gain-of-function mutations in the gene can cause an extreme pain disorder.^{14,15}

Action potentials travel through the afferent, nociceptor axon and reach the soma (cell body), which is found in the dorsal root ganglion (DRG). The soma has central terminals synapsing in the superficial laminae (I and II) of the spinal dorsal horn. Circuits within the spinal cord process sensory input and relay them to brain centers via multiple neural tracts. The lateral spinothalamic tract projects to the lateral thalamus where the discriminative and sensory components of pain are processed. The medial aspect of the spinothalamic tract and the spinoparabrachial tract project to the medial thalamus and limbic structures, such as the anterior cingulate cortex (ACC) and central nucleus of the amygdala, which are thought to mediate the emotional and aversive aspects of pain.⁷ Ultimately, pain is perceived in the cerebral cortex and a response is generated, such as a cortico-spinal signal to activate withdrawal from the noxious stimulus.^{7,16} Of note, reflexive withdrawal mechanisms triggered at the level of the spinal cord sometimes take place as well.¹⁶

Pathologic Pain

While pain is essential for physiologic functioning, it can become maladaptive in pathological conditions such as inflammation, cancer, neuropathy, viral infections, and diabetes.⁷

Chronic pain can persist long after an acute injury has taken place or arise without any clear pathological trigger. Patients often complain of spontaneous, ongoing pain that may be different in mechanism from the stimuli that evoked the initial pain.⁷ They also commonly experience hyperalgesia and allodynia in the chronic pain region. Hyperalgesia constitutes increased sensitivity to painful stimuli (i.e., noxious stimuli produce greater pain than usual). Allodynia refers to painful sensations in response to stimuli that are typically not painful. Recent evidence suggests that these chronic changes reflect endogenous sensitization over time of the peripheral and central neural structures that mediate pain.⁷

Peripheral Sensitization in Chronic Pain

Peripheral sensitization appears to play a role in many chronic pain conditions. This manifests as a reduction in the *threshold* of nociceptor reactivity or as an increase in *magnitude* of reactivity.³ These changes are induced by molecules released at the site of inflammation by nociceptors and non-neuronal cells, such as mast cells, macrophages, and neutrophils. These chemical mediators influence nociceptor excitability at the transcriptional or post-translational level and include prostaglandins, protons, ATP, leukotrienes, and growth factors. For instance, nerve growth factor (NGF) and granulocyte-macrophage colony stimulation factors (GM-CSF) recruit downstream enzymes such as phospholipase C (PLC) and mitogen-activated protein kinase (MAPK), which activate transducer molecules such as Nav1.8 and TRPV1, increase protein transcription rates, and ultimately raise nociceptor excitability.^{8,17,18} Peripheral sensitization in chronic pain is also mediated by sensitization in the dorsal root ganglia. For instance, in chronic neuropathic pain, neutrophils and T cells have been shown to invade DRG somata of nociceptors, which become a source of ectopic pain discharges.¹⁹

Central Sensitization in Chronic Pain

In addition to peripheral sensitization, chronic pain involves sensitization of pain-related structures within the central nervous system.³ The synaptic communication between primary afferent neurons and secondary afferent (spinal) neurons is largely mediated by glutamate. Thus glutamatergic receptors, which are modulated by neuropeptides such as substance P, play a significant role in determining the strength of synaptic transmission and ultimately the strength of pain sensation. In chronic pain, both pre-synaptic and post-synaptic mechanisms lead to long-term potentiation (LTP) of synapses in the spinal dorsal horn, increasing excitability of spinal neurons and therefore raising pain sensitivity at the CNS level.^{20,21} For instance, the persistent nociceptive activity leading up to chronic pain causes insertion of more glutamate receptors in postsynaptic membranes and production of cyclooxygenases and prostaglandins that facilitate greater synaptic neurotransmitter release.⁷ This activity also drives expression throughout the nervous system of genes responsible for producing pain-related proteins such as TRPV1, COX-2, and calcium channels.^{22,23}

Beyond these molecular changes, structural and connectivity-related changes also take place in the CNS during chronic pain states. Studies have demonstrated decreases in gray matter volume of the anterior cingulate cortex and dorsolateral prefrontal cortex, altered brain network connectivity in circuits involving cognition and autonomic responses, and changes in nerve fiber tracts.^{7,24-26} These widespread changes lead to altered moods and behaviors, including spontaneous pain at rest, anxiety and depression, decreased attention and worsened emotional state.² Thus, chronic pain involves sensitization and other changes not only in the peripheral nervous system, but in the central nervous system as well.

Challenges in Pharmacologic Treatments for Pain

Most pain medications used today, such as acetaminophen, opioids, salicylic acid, gabapentin, and non-steroidal anti-inflammatory drugs, target specific mechanisms in pain generation that been known for years. These drugs often have limited ability to alleviate pain, particularly chronic pain, and the most effective forms can cause substantial side effects such as nausea, constipation, cardiovascular complications, respiratory depression, and addiction.³ Despite great advances in our understanding of the neurophysiology underlying acute and chronic pain, there remain major limitations in our development of new pharmacologic treatments. One challenge is that many molecular mediators involved in pain processing at the central and peripheral levels, such as PLC and MAPK, are involved in numerous physiologic processes and can therefore not be targeted. Another challenge involves the multiple redundant mechanisms in pain pathways which prevent us from treating pain with a single drug target.³

These limitations have led researchers to examine endogenous mechanisms by which pain is inhibited and develop new exogenous tools for treating pain through modulation of central nervous system activity.

Endogenous Pain Modulation: The Role of Descending Noxious Inhibitory Controls

Recent literature suggests that neural transmission of pain signals from the periphery is influenced by an endogenous pain regulatory system.^{7,27,28} This pain modulatory system spans the nervous system, involving cortical (e.g., prefrontal cortex, cingulate cortex, and insular cortex), midbrain, and brainstem regions (e.g., periaqueductal gray (PAG), rostroventral medulla (RVM), and subnucleus reticularis dorsalis (SRD) of the medulla).^{7,29} Descending output from these regions project down the spinal cord and can inhibit or facilitate sensory processing in the spinal dorsal horn.^{7,29}

Diffuse noxious inhibitory controls (DNIC) refers to a spinal-medullary-spinal pathway within the endogenous pain regulatory system that decreases sensitivity to subsequent pain after exposure to an initial noxious stimulus, often summarized as “pain inhibits pain.”^{27,30} Recently termed “conditioned pain modulation,”³¹ DNIC can be examined in laboratory settings by administering a noxious stimulus (the “test-stimulus”) after introducing a heterotopic conditioning stimulus (the “conditioning-stimulus”).²⁷ When pain signals ascend through the spinal cord from the periphery, supraspinal structures responsible for DNIC (such as the SRD of the caudal medulla) respond by triggering descending inhibition of lamina I neurons in the spinal dorsal horn.^{27,32-34} This inhibition increases the threshold at which pain stimuli are transmitted and ultimately perceived. Brainstem regions involved in DNIC such as the SRD are influenced by cortical regions such as the prefrontal cortex, explaining why psychological factors seem to influence DNIC responses.³⁴

Endogenous pain regulatory pathways such as DNIC serve several adaptive roles. For instance, they allow an organism to suppress pain during dire circumstances to help achieve survival.⁷ They also play an important role in inhibiting the processes that lead to central sensitization found in chronic pain.⁷ Indeed, patients with hypersensitivity to pain and chronic pain have been observed to possess impaired DNIC responses.^{27,35-39} Interestingly, impaired DNIC response on pre-operative testing has been associated with a greater risk of chronic post-thoracotomy pain.⁴⁰ Endogenous pain modulation may also provide a mechanistic basis for placebo-induced analgesia.⁴¹ Finally, some have theorized that non-pharmacologic pain therapies such as acupuncture and transcutaneous electrical nerve stimulation may contribute to analgesia, in part, through activation of DNIC.^{7,42-44}

Thus DNIC is a pain regulatory mechanism that endogenously influences the perception of pain. It is found to be dysfunctional in chronic pain states and may be involved in non-

pharmacologic pain therapies. In the next section, we will turn to a potential tool for exogenously modulating pain known as transcranial direct current stimulation. First, I will introduce the field of non-invasive brain stimulation generally, then discuss the neurophysiology of tDCS in particular, and finally discuss the potential use of tDCS in modulating pain.

Brain Stimulation: A Rapidly Growing Field

Brain stimulation techniques and applications have been rapidly increasing in neuroscience and clinical medicine. The most invasive of these techniques, deep brain stimulation, is a method that permits direct and precise stimulation of deep structures in the brain such as thalamic, subthalamic, and pallidal nuclei. This technique has been used to treat dystonias in Parkinson's disease⁴⁵ and holds potential for helping those with obsessive compulsive disorder⁴⁶ and mood disorders.⁴⁷ At the cortical level, electrodes placed directly on the dura to stimulate the motor cortex have been shown to alleviate chronic neuropathic pain.⁴⁸ These methods of stimulation are limited, however, by the need to surgically penetrate the skull, a costly endeavor with significant medical risks.

In part due to the above limitations, non-invasive transcranial stimulation techniques have been developed which allow for modulation of brain activity at a lower cost and with considerably less risk. Perhaps the most well known of these techniques is transcranial magnetic stimulation (TMS), which was developed in 1985 and applies a short-lasting magnetic field to stimulate electrical currents and action potentials in the underlying cortex (See Figure 1).⁴⁹⁻⁵¹

Among the many brain stimulation techniques available, tDCS is one of the simplest in design, involving administration of weak direct current straight through the scalp.^{51,52} In its most common form, two sponge-based electrodes are attached to a battery-powered current generator that delivers weak current (usually below 10 mA). The electrodes are soaked in saline and held

on two separate parts of the scalp with a rubber band that is placed around the head (See Figure 2). Current traveling through the circuit enters the scalp and skull, reaching the underlying cortex and inducing neurophysiological effects. In contrast to TMS, which triggers action potentials, tDCS solely modulates neuronal excitability at subthreshold levels and therefore does not directly induce action potentials. Of note, electroconvulsive therapy is similar to tDCS in design but utilizes substantially greater current intensities (>500 mA vs. less than 10mA for tDCS).⁵³

Importantly, TMS has provided an important window into the neurophysiology underlying the effects of tDCS. TMS can be used to induce motor-evoked potentials (MEPs), for instance, by application of TMS to the motor cortex. Since MEP amplitude is correlated with motor cortex excitability, TMS can therefore be used to examine the effects of transcranial direct current stimulation on motor cortex excitability.^{50,54}

Neurophysiology of tDCS

At the turn of the millennium, Nitsche and Paulus demonstrated that tDCS can be used to modulate cortical excitability.⁵⁵ Using TMS-induced MEPs as a marker of motor cortex excitability, they found that application of weak direct current to the scalp was associated with changes in cortical excitability up to 40% that lasted several minutes to hours beyond the end of stimulation.⁵⁵ Importantly, they showed that electrode montage was essential in determining tDCS effects. Later studies revealed that while about half of tDCS current diffuses across the scalp, sufficient current penetrates the scalp and skull to influence transmembrane neuronal potentials and modulate neuronal excitability in the cortex without eliciting action potentials.⁵⁶⁻⁵⁸

The immediate effects of tDCS are due to modulation of neuronal membrane potentials at subthreshold levels, which increases or decreases the rate of action potential firing. On a cellular level, the voltage gradient between electrodes establishes opposing polarities at either end of the

neurons in the electric field. This generates a difference in the transmembrane potential of neuronal membranes and thereby causes current to flow across the membrane and through the neuron in accordance with resistance properties of the membrane and intracellular space⁵⁹. This flow of current modulates the neuronal membrane potential and results in altered spontaneous neuronal activity. Usually, anodal stimulation will depolarize membranes to subthreshold levels and increase cortical excitability, while cathodal stimulation will hyperpolarize membranes and decrease cortical excitability.⁶⁰⁻⁶² Ultimately, the direction of polarization and changes in excitability will depend on the orientation of axons and dendrites in the induced electric field. Radman and colleagues, for instance, demonstrated that the neural somata in cortical layers V and VI are most susceptible to tDCS effects.⁶³

Changes in cortical excitability are dependent on a number of parameters such as stimulation duration and current density, with greater durations and larger current densities having greater and longer-lasting effects.^{55,64-68} (See Table 1 for summary of tDCS parameters and related effects). Electrophysiologic and TMS studies have shown that tDCS can modulate excitability of cortical areas immediately beneath the electrodes as well as distant areas connected to the initial area of stimulation in a polarity-dependent fashion.^{69,70} Similarly, fMRI and EEG studies reveal that although tDCS has its strongest effects on the underlying cortex, the stimulation can provoke widespread and sustained changes in other brain regions as well.^{69,71,72} More recently, tDCS has been observed to influence spinal cord excitability.^{73,74} Therefore, clinical effects of tDCS on conditions such as chronic pain might be mediated by influences on many regions in the central nervous system.

Neuropharmacological studies suggest that the long-term effects of tDCS, which can last well beyond stimulation, likely involve NMDA-receptor dependent mechanisms.⁷⁵ Furthermore,

functional near-infrared spectroscopy has shown that tDCS can lead to increased cerebral blood flow to the stimulated region.⁷⁶

Scientific and Clinical Uses of tDCS

In recent years, tDCS has been tested for a wide array of scientific and clinical purposes. For instance, researchers have utilized it to examine the effects of cortical modulation on language,⁷⁷ decision-making,⁷⁸ emotional pain,⁷⁹ sensory perception,⁸⁰ and memory.⁸¹ Clinically, tDCS has been proposed as a new tool for enhancing motor and memory rehabilitation following stroke.⁸²⁻⁸⁵ It is also being tested for modulating mood and cognitive processes such as craving in substance abuse.⁸⁶⁻⁸⁸

Exogenous Pain Modulation Using tDCS

Primary motor cortex stimulation using tDCS may also be an effective tool for alleviating chronic pain. This approach finds theoretical support in the neurosurgical literature, where an abundance of evidence suggests that invasive primary motor cortex stimulation can significantly reduce chronic pain, with a weighted responder rate of over 70%.⁴⁸ While the precise mechanism of analgesia is unclear, growing evidence suggests that motor cortex stimulation triggers rapid phasic activation in the lateral thalamus, which results in modulation of activity in other pain-related regions such as the medial thalamus, ventrolateral thalamus, insula, anterior cingulate gyrus, and upper brainstem (e.g., periaqueductal gray matter).⁸⁹⁻⁹¹ More specifically, lateral thalamic modulation leads to inhibition of thalamic sensory neurons, cingulate modulation leads to decreased emotional appraisal of pain, and periaqueductal gray modulation leads to descending inhibition toward the spinal cord (similar to DNIC).^{91,92} Evidence suggests motor cortex stimulation may also cause endogenous opioid release and directly inhibit the

somatosensory cortex.^{91,93} The motor cortex thus appears to be an “entry port” for modulating deep brain structures, with downstream modulatory effects on pain regions in the brainstem, limbic system, and spinal cord.⁴⁸

Given the positive results with invasive motor cortex stimulation, researchers have examined whether non-invasive stimulation methods such as tDCS may work as well. Indeed, tDCS studies have shown positive results in reducing chronic pain in patients with traumatic spinal cord injury,⁹⁴ terminal cancer,⁹⁵ fibromyalgia,⁹⁶⁻⁹⁸ chronic migraine,⁹⁹ refractory oral pain,¹⁰⁰ and chronic pelvic pain.¹⁰¹ A 2010 Cochrane review and more recent 2012 independent review reported that motor cortex stimulation with tDCS does have short-term effects on chronic pain, but remarked that the data is insufficient to draw firm conclusions.^{102,103}

Combining Techniques for Modulating Pain

Growing literature reveals that tDCS as a modulatory technique may be more effective when administered in conjunction with other therapeutic modalities. For instance, tDCS coupled with visual illusion therapy and transcutaneous electrical nerve stimulation (TENS) has been found to be more efficacious in treating chronic pain.^{104,105} Likewise, in the setting of motor rehabilitation following stroke, outcomes are enhanced when tDCS is combined with constraint-induced movement therapy.¹⁰⁶ These observations suggest that combination of tDCS with activation of endogenous DNIC inhibitory pathways may yield similarly enhanced effects on pain modulation.

Magnetic Resonance Spectroscopy of Pain

Proton magnetic resonance spectroscopy (¹H-MRS) is a non-invasive magnetic resonance imaging technique that can measure concentrations of metabolites, such as N-acetylaspartate

(NAA), in the human brain. MRS has long been utilized to study neurochemical changes in patients with neurological and psychiatric disorders, including intracranial neoplasm, stroke, depression, and schizophrenia.¹⁰⁷ Abnormalities in metabolite levels have been linked to pathological changes in neural tissue. For instance, NAA concentration has been viewed as a marker of neural density, viability, and overall function, and decreases may suggest impaired neural function.¹⁰⁸⁻¹¹⁰ In recent years, researchers have used MRS to examine metabolite changes in patients with chronic pain.¹¹¹ Interestingly, studies have found decreased levels of NAA in the thalamus in patients with chronic neuropathic pain,¹¹² trigeminal neuralgia,¹¹³ and migraine.¹¹⁴ Glutamate is an excitatory neurotransmitter known to be involved in pain, and pain relief in chronic pain patients has been associated with decreasing glutamate-glutamine levels in the insular cortex (glutamine is a precursor to glutamate).¹¹⁵ In contrast, gamma-aminobutyric acid (GABA) is a key inhibitory neurotransmitter involved in pain, and chronic pain patients have been shown to have low GABA levels in the insular cortex and to be more sensitive to experimental pain.¹¹⁶ These observations suggest that MRS might be useful as a diagnostic tool in chronic pain or as a predictor of response to various therapeutic interventions.

Specific Aims of the Present Study

The following aims and hypotheses were set forth for the present study:

Aim 1: Our primary aim was to compare exogenous (tDCS) and endogenous (DNIC) pain modulation approaches and determine whether combining them synergistically increases the threshold at which pain is perceived in healthy subjects. The primary outcome was change in pain perception threshold, which was measured in pounds of pressure required to elicit a pain response. We hypothesized that these two modulation techniques would significantly increase pain thresholds when administered independently and that they would have significant

additive or synergistic effects when combined. *Please Note: The terms “pain threshold” and “pressure-pain threshold (PPT)” will be used throughout to refer to pain perception threshold (i.e., the threshold at which subjects begin to perceive pain caused by mechanical pressure). Pain tolerance threshold (i.e., the threshold at which subjects can no longer tolerate pain) is an outcome that was not examined in this study for ethical and subject recruitment reasons.*

Aim 2: Our secondary aim was to determine whether active tDCS alters the threshold for sensory perception as compared with sham tDCS. This outcome (“sensory threshold”) was measured as the smallest von Frey monofilament thickness necessary for perceiving sensation. *This aim was exploratory in nature and we did not power our study for this aim.*

Aim 3: Our final aim was to determine whether baseline neurochemical concentrations in the motor cortex, thalamus, and anterior cingulate cortex significantly correlate with subject responses to the pain modulatory interventions. We predicted that lower baseline glutamate and glutamine levels in our regions of interest would be associated with greater pain threshold increases following stimulation (and that this association would not be observed in the occipital cortex, which was our control region). This hypothesis follows from a recent study that found decreased baseline levels of glutamate-glutamine to be positively correlated with chronic pain improvement following non-invasive brain stimulation.¹¹⁷ *This aim was exploratory in nature and we did not power our study for this aim.*

Methods

Experimental Design

We performed a double-blinded, randomized, placebo-controlled trial with a crossover design to investigate the effects of tDCS and DNIC on pain thresholds in healthy human subjects. The protocol for the investigation was approved by Spaulding Rehabilitation Hospital's institutional review board. Prior to participation, the subjects provided written, informed consent.

Participants

We recruited subjects between the ages 18 and 64 (inclusive) from the greater Boston area using online listings and flyers. Criteria for exclusion included the following: chronic pain symptoms in the past six months, history of psychiatric or neurological disorders, substance abuse history in the past six months, routine use of prescription drugs, pregnancy, and presence of tDCS or MRI contraindications (e.g., implanted brain medical devices; see Appendix A). We chose conservative estimates in our sample size calculations. Assuming alpha of 5% and beta of 20% (power = 80%), if we were to use 15 subjects in this crossover experiment, we calculated that we could detect differences between groups of 0.45 using two-tailed t-tests. This is a smaller effect size than in a previous study performed in our lab¹¹⁸ and was therefore deemed sufficient for examining our hypothesis. This study included 15 participants (mean age 36.7 ± 11.0 years, 9 females). The subjects and rater were all blinded to the stimulation conditions (active vs. sham tDCS).

Study Sequence

The study took place over the course of three visits. During Visit 1, participants underwent magnetic resonance spectroscopy to assess baseline concentrations of metabolites in

specific brain regions. Within three days of Visit 1, subjects were randomized by blocks of four subjects to receive either active or sham tDCS (Visit 2). During this session, tDCS administration was preceded and followed by sensory and cognitive assessments. Seven or more days after Visit 2, subjects experienced identical procedures as in Visit 2, with the only difference being that conditions of stimulation were interchanged (sham or active tDCS, respectively). A diagram of the experimental design is presented in Figure 3.

Magnetic Resonance Spectroscopy

We scanned each subject using MRS to assess the baseline levels of glutamate and other neural metabolites in brain regions of interest related to pain. ¹H-MRS was performed with a Philips Achieva 3.0T (Philips Healthcare, Best, Netherlands) that ran Release 2.6 software. Subjects were asked to remain still for the entirety of data collection, which lasted about 30 minutes. We acquired single-voxel proton MR spectra and employed LCModel (Stephen Provencher Inc., Oakville, Ontario, Canada) to assess ratios of metabolite concentration. We positioned the MRS voxels ($2 \times 2 \times 2$ cm, 8 cm^3) on the sagittal, axial, and coronal images, targeting as regions of interest the thalamus, motor cortex, and anterior cingulate cortex (Brodmann area 24).^{119,120} Data was also collected from the occipital cortex, which served as the control region. We acquired spectra using a point-resolved spectroscopy (PRESS) sequence with a spectral width of 5000 Hz, short echo time (TE) of 35 milliseconds, repetition time (TR) of 2 seconds, 2048 time points, and partial water suppression. Manufacturer-supplied shimming procedures were performed.

We analyzed metabolite concentrations using LCModel (Stephen Provencher Inc., Oakville, Ontario, Canada). We determined levels of glutamate (Glu), glutamine (Gln), N-acetylaspartate (NAA), N-acetylaspartateglutamate (NAAG), NAA+NAAG (total NAA),

creatine (Cr), myoinositol (mI), and choline by fitting a linear combination of a basis set of metabolite model spectra to the data. We analyzed the spectrum set from 0.2ppm up to 3.8ppm with no water scaling or eddy-current correction. The metabolite concentrations were reported in terms of mM and ratios relative to the Cr peak. For each subject, the metabolite concentrations and metabolite-to-creatine ratios were determined in the acquired spectra.

Transcranial Direct Current Stimulation

We administered direct current (DC) to the scalp using rubber electrodes enclosed in saline-soaked sponges (35 cm²). Rubber bands were used to hold the electrodes in place on the scalp and the electrodes were connected by wires to a battery powered DC generator (Activa Dose, Salt lake City, UT). The anode electrode was positioned on the scalp just above the left primary motor cortex (M1) and the cathode was placed on the right forehead above the right supra-orbital area. This montage seems to be optimal for achievement of pain modulation.⁹⁷ We localized M1 using the electroencephalogram (EEG) 10/20 system used in previous studies⁹⁴ and confirmed correct localization through magnetic resonance imaging (MRI) during the MRS scan (Visit 1) by placing a Vitamin E on the scalp above the expected M1 location. The electrode sponges covered a large portion of the motor cortex, including the segments that mediate activity in the upper limb and parts of the lower limb and face, as in Fregni et al. (2006).⁹⁴ This placement has been shown to increase excitability in M1.⁵⁵

During active stimulation conditions, we administered 2 mA of anodal tDCS to M1 for 20 minutes in accordance with current stimulation protocols.¹²¹ At the beginning and end of stimulation, current level was gradually increased and decreased over the course of 10 seconds in order to avoid visual sensations and other side effects. During sham stimulation conditions, identical protocols were used, but tDCS current was only administered for the first 30 seconds of

the 20-minute session. Previous literature has demonstrated that application of current for 30 seconds is a valid method of blinding and that application of current for under 3 minutes does not influence cortical excitability.^{55,122,123} Subjects receiving active and sham stimulation typically feel an itching sensation on the scalp beneath each electrode at the start of stimulation that wanes over time. Of note, studies have shown that a single session of active tDCS using 2 mA current is safe in non-pregnant, healthy adults, with only minor and short-lasting adverse effects.^{68,124}

Assessments

During Visits 2 and 3, a rater (J.S.R.) who was blinded to stimulation conditions performed all assessments. These assessments took place immediately before and after the 20-minute active or sham tDCS sessions and were administered in the following order (explanations below):

- (1) Visual Analog Scale for Anxiety
- (2) Beck Depression Inventory (only administered before stimulation)
- (3) Von Frey Hair Assessment
- (4) Pressure-Pain Threshold Assessment
- (5) Pressure-Pain Threshold Assessment During Cold-Water Immersion (DNIC)
- (6) Trail-Making Tests A & B
- (7) Stroop Test
- (8) Simple Reaction Time Test

Following stimulation, we immediately administered two questionnaires to assess the occurrence of adverse effects and determine the success of our blinding procedures. We then repeated all of the assessments listed above (excluding the Beck depression inventory).

Sensory Assessments

As discussed above, our primary aim was to evaluate the influences of tDCS and DNIC on the threshold for perceiving pain. We secondarily aimed to determine whether these modulatory approaches affect the threshold for perceiving sensation.

Von Frey hair (VFH) assessment. We measured subjects' thresholds for perceiving sensation of mechanical pressure using von Frey hair monofilaments with increasing intensities (sizes 1.65 to 6.65, which corresponds to target forces of 0.008 grams to 300 grams).¹²⁵ While the subject's eyes were closed or averted, we applied increasingly thick monofilaments to the thenar region of the subject's right hand until the subject reported sensing the tactile stimulus. The monofilament thickness required to elicit this response was recorded as the sensory threshold.

Pressure-Pain Threshold (PPT). Pain thresholds (i.e., the threshold at which subjects perceive pain) were assessed by applying an increasing amount of blunt pressure using the 1-cm² hard-rubber end of an FDA-approved pain threshold assessment device (Commander Algometer, JTECH Medical, Salt Lake City, UT). Higher pain thresholds signify lower sensitivity to painful stimuli. Pressure was increasingly applied to the thenar region of the right hand at a rate of 2 pounds per second until the subjects reported a sensation of pain, at which point the device was promptly removed. The pounds of pressure required to elicit a sensation of pain was recorded as the pressure-pain threshold, or PPT. This assessment was repeated 3 times.

Pressure-Pain Threshold during Cold-Water Immersion. We assessed the degree to which pain perception is modulated by DNIC by measuring PPT during immersion of the contralateral hand in cold water. In this paradigm, the cold-water immersion was the conditioning-stimulus (CS) and the blunt algometric pressure was the testing-stimulus (TS). Subject placed their left hands into cold water (10-12°C) for a total of one minute. During the final 30 seconds of cold-

water immersion, the PPT assessment was performed on the right hand. In a few cases, the subjects found 12°C too cold and the water temperature was therefore increased to more tolerable, but still reportedly painfully cold, levels. The water temperature was kept constant for each subject across the experiment.

Assessment of Potential Confounders

Levels of anxiety and depression were assessed because these variables can be significant confounders for variations in pain perception.

Visual Analog Scale (VAS) for Anxiety. Subjects rated their current level of anxiety on a visual scale from 0 to 10 (see Appendix B).

Beck Depression Inventory (BDI). Subjects completed 21 multiple-choice questions to determine the presence and extent of depression symptoms.

Cognitive Assessments

To monitor for potential adverse effects of tDCS on cognitive function, we administered the following cognitive assessments: *Trail-Making Tests A & B* (assesses attention and working memory function; see Appendix C), *Stroop Test* (assesses selective attention and interference proclivity as well as executive function), and *Simple Reaction Time Test* (assesses attention and reaction time using software conducted with Superlab pro v2.0 software (Cedrus Corporation, San Pedro, CA)). During 30 trials, subjects were asked to push a response key as soon as they saw a 4-cm circle appear on the computer screen. This occurred randomly at 2 to 5 second intervals. Response times were recorded and no feedback was provided to subjects).

Side effects and Blinding Assessments

Safety Assessment. Immediately following tDCS administration, subjects completed a questionnaire (Appendix D) inquiring about the occurrence of any side effects such as headache, scalp pain, tingling, neck pain, scalp burns, skin redness, trouble concentrating, sleepiness, and acute mood change.

Blinding Assessment. Subjects were asked to guess whether they had received active or sham tDCS during the session and provide a rating of their level of confidence that they are correct (Appendix E).

Statistical analysis

We analyzed data using Stata[®] statistical software (version 9.1, College Station, Texas). We conducted a Shapiro-Wilk test for normality. We then ran a repeated-measures analysis of variance (ANOVA) in which the dependent variable was change in pain threshold (as measured in pounds of algometric pressure). The independent variables included in the ANOVA were condition (active tDCS, sham tDCS, DNIC, combined active tDCS and DNIC) and the random variable subject ID to control for within subject variability. We performed post-hoc comparisons using two-tailed, paired t-tests and corrected for multiple comparisons when appropriate. We also used two-tailed paired t-tests to evaluate the effects of active and sham tDCS on sensory perception (VFH), level of anxiety (VAS), and cognitive performance (Trail-Making A & B, Stroop test, and Simple Reaction Time). Finally, Pearson correlation coefficients were computed to determine the relationships between baseline brain metabolite concentrations and changes in pain threshold across the varying experimental conditions. We considered these secondary, correlational analyses exploratory and therefore did not make Bonferroni corrections (we set significance level to $p = 0.05$).

Results

Side effects, Blinding, and Test for Normality

Fifteen participants (mean age 36.7 ± 11.0 years, 9 females) were included in our analysis. The study procedures were tolerated well by subjects and no significant adverse effects were reported. There were no significant differences in adverse effects reported by subjects when comparing active and sham tDCS sessions. Table 2 summarizes the frequency of side effects reported following active and sham tDCS. The data was analyzed for potential order effects by comparing side effects in those who received active tDCS during the first stimulation session and those who received sham tDCS during that session. While there appeared to be order effects for various side effects, these order effects were not associated with stimulation condition. For example, subjects reported greater occurrence of tingling in the first session regardless of the stimulation condition (subjects receiving active-followed-by-sham tDCS reported frequencies of 83% vs. 50%, respectively, while subjects receiving sham-followed-by-active tDCS reported frequencies of 78% and 67%, respectively). For other adverse effects such as sleepiness there did not appear to be an order effect (the same analysis showed 33% vs. 33% and 44% vs. 33%, respectively). On the blinding assessments, 5 subjects (33%) correctly guessed whether they received active or sham tDCS during both sessions and the other 10 subjects did not. This guessing success was at the level of chance ($p=0.2$, comparison between correct and incorrect guesses), suggesting that our protocol for sham stimulation was sufficient for blinding subjects. The Shapiro-Wilk test for normality suggested that the data was normally distributed. In fact, for our main outcome (PPT), our results obtained a W score of 0.97 ($p=0.18$).

Changes in Pressure-Pain Threshold

We analyzed whether stimulation condition was associated with an altered threshold for pain perception using a repeated-measures ANOVA. In this model, change in pain threshold (in units of pounds) was the dependent variable and the independent variables were condition (active tDCS, sham tDCS, DNIC, combined active tDCS and DNIC) and the random variable subject ID (allowing us to control for variability within subjects). The ANOVA showed a significant difference in outcomes between conditions ($F_{(3,42)}=8.12$; $p<0.001$).

We next performed post hoc analyses to compare the effects of the varying modulation conditions on pain thresholds. Two-tailed paired t-tests showed significant increases in pain threshold after active tDCS compared to sham conditions ($p<0.05$), after DNIC compared to sham conditions ($p<0.005$), and after combined active tDCS and DNIC compared to sham conditions ($p<0.005$) (See Figure 4). We did not observe a significant difference when comparing changes in pain threshold following active tDCS to changes in pain threshold following DNIC ($p=0.35$), suggesting that the pain modulation effects of these approaches as administered in our paradigm are comparable. Pain threshold increase following combined active tDCS and DNIC was significantly greater than following DNIC alone ($p<0.01$). While the pain threshold increase following combined active tDCS and DNIC was greater in magnitude than following active tDCS alone, this difference did not reach levels of significance ($p=0.19$).

Of note, we found that subject responses to tDCS alone appeared to be associated with responses to combined tDCS and DNIC. Indeed calculation of Pearson's correlation coefficient revealed that increases in pain threshold after active tDCS were positively and significantly correlated with increases after combined active tDCS and DNIC ($r=0.54$, $p<0.05$). This may suggest that both interventions share similar predictors of response.

Changes in Sensory Threshold

Von Frey hair test results were examined using repeated-measures ANOVA and two-tailed paired t-tests. Change in thresholds for perceiving the sensory stimulus ($\text{Threshold}_{\text{after}} - \text{Threshold}_{\text{before}}$) increased significantly following active tDCS compared to following sham tDCS ($F_{(1,14)}=5.88$, $p<0.05$; 0.29 ± 0.43 filament units increase following active tDCS vs. 0.00 ± 0.37 filament units increase following sham tDCS, $p<0.05$).

Behavioral and Cognitive Assessment Results

There were no significant differences in subject performance on the Beck depression inventory when administered prior to the active stimulation session compared to the sham stimulation session (1.20 ± 2.14 vs. 1.27 ± 2.49 , $p=0.86$). Average VAS level of anxiety reported before stimulation (regardless of whether it was active or sham) was significantly higher than after stimulation (1.13 ± 1.48 VAS units before vs. 0.83 ± 1.14 VAS units after, $p<0.05$). Changes in reported anxiety were not significantly different following active stimulation compared to following sham stimulation. We did not observe significant differences in cognitive test performance after active tDCS compared to after sham tDCS.

Brain Metabolite Concentration and Pain Modulation Response

We performed exploratory post-hoc, correlational analyses to examine whether baseline concentrations of brain metabolites were associated with responses to the varying experimental conditions. We found that increase in pain threshold after active tDCS was positively correlated with total-NAA concentration in the anterior cingulate cortex ($r=0.58$, $p<0.05$), negatively correlated with Gln concentration and Gln/Cr in the thalamus ($r=-0.60$, $p<0.05$; $r=-0.61$, $p<0.05$, respectively), and positively correlated with mI concentration in the anterior cingulate cortex and

occipital cortex ($r=0.66$, $p<0.01$; $r=0.52$, $p<0.05$, respectively). Pain threshold increase following sham tDCS was negatively correlated with Gln concentration in the anterior cingulate cortex, motor cortex, and occipital cortex ($r=-0.53$, $p<0.05$; $r=-0.59$, $p<0.05$; $r=-0.54$, $p<0.05$, respectively) and Gln/Cr in the motor cortex ($r=-0.67$, $p<0.01$). Pain thresholds measured during cold-water immersion (DNIC) prior to active stimulation were negatively associated with Glu and Glu/Cr in the occipital cortex ($r=-0.54$, $p<0.05$; $r=-0.59$, $p<0.05$, respectively). Pain thresholds after active tDCS administration were negatively correlated with Glu/Cr in the occipital cortex ($r=-0.53$, $p<0.05$) and positively correlated with mI in the anterior cingulate cortex ($r=0.58$, $p<0.05$).

Discussion

Increase in Pain Thresholds

In this investigation we aimed to compare exogenous (tDCS) and endogenous (DNIC) pain modulation approaches and determine whether their combination can result in synergistic effects on pain perception in healthy human subjects. We found that when administered alone both tDCS and the DNIC-induction paradigm significantly increased the thresholds at which subjects perceive pain compared to sham conditions. Interestingly, the pain threshold increases observed following tDCS alone and DNIC alone were not significantly different in magnitude. Furthermore, when combined, these modulatory approaches appeared to have additive effects in raising pain thresholds. Activation of DNIC in conjunction with tDCS increased pain thresholds significantly greater than activation of DNIC alone. Thus, tDCS and DNIC appear to have additive, and possibly synergistic, effects. This observation is consistent with previous literature that found increased efficacies of pain and motor rehabilitation therapies when administered in combination with tDCS.¹⁰⁴⁻¹⁰⁶

From a theoretical point of view, there are a number of possible ways to explain the mechanistic underpinnings of our findings. It is plausible that the observed additive effect of these combined modulatory approaches results from influences on two distinct regions of neural activity mediating pain. It is also possible that tDCS and DNIC in fact influence similar pain-related regions, thus having additive effects by increasing the modulatory “dose” administered to these regions. A third mechanism might involve one modulatory approach synergistically *potentiating* the other. For example, motor cortex stimulation with tDCS might facilitate activity in DNIC-related neural networks and therefore enhance their endogenous effects on pain, or vice versa.

From a neurophysiological perspective, each of these theories is plausible and may help explain our observed findings. tDCS and DNIC appear to influence pain perception through different but partially overlapping neural pathways. Primary motor cortex stimulation with tDCS likely modulates pain through direct effects on ventral lateral and anterior thalamic nuclei (via cortico-thalamic pathways), which result in secondary modulatory effects on other pain-related regions such as the medial thalamus, anterior cingulate cortex, insula, and periaqueductal gray matter of the brainstem.^{69,89-91,94} More specifically, lateral thalamic modulation induces inhibition of thalamic sensory neurons, cingulate modulation decreases emotional appraisal of pain, and periaqueductal gray modulation leads to descending inhibition toward the spinal cord.^{91,92} Motor cortex stimulation may also cause endogenous opioid release and directly inhibit the somatosensory cortex.^{91,93}

Pain modulation in DNIC is different from tDCS in that it begins with stimulation of the peripheral nervous system rather than CNS tissue, but it may ultimately influence some overlapping pain-related regions. In DNIC, pain signals ascend through the spinal cord from the periphery and ultimately influence brainstem structures such as the subnucleus reticularis dorsalis of the caudal medulla which triggers descending inhibition of lamina I neurons in the spinal dorsal horn.^{27,32-34,126} Similar to motor cortex stimulation, DNIC may also involve descending inhibition from periaqueductal gray matter, endogenous opioid release, and modulation of primary somatosensory cortical activity.¹²⁷ Thus, DNIC and motor cortex stimulation with tDCS may modulate pain through influences on distinct regions (e.g., lateral thalamus with tDCS; SRD with DNIC) or overlapping regions (e.g., periaqueductal gray matter with both). Finally, it is possible that one of these approaches synergistically *potentiates* the other. For instance, neurons of the subnucleus reticularis dorsalis receive massive corticofugal

projections and are modulated by the cingulate cortex, potentially explaining how cortical stimulation with tDCS could potentiate DNIC effects.^{128,129}

As mentioned earlier, this experiment is consistent with a growing literature that suggests various pain and motor rehabilitation therapies are more effective when administered in conjunction with tDCS. For instance, combinations of transcutaneous electrical nerve stimulation and visual illusion therapy with tDCS have been found to treat chronic pain more effectively.^{104,105} Similarly, in the setting of motor rehabilitation after stroke, outcomes are improved when tDCS is joined with constraint-induced movement therapy.¹⁰⁶ While this investigation involved healthy subjects, our results suggest that future work should examine whether the combination of tDCS and DNIC-related pain modulation might help in treating chronic pain patients. Fibromyalgia patients, for instance, have been found to have dysfunctional endogenous pain inhibition (DNIC)³⁷ and this deficiency has been associated with reduced activation of the rostral anterior cingulate cortex and associated brainstem regions, both of which play important roles in the endogenous pain regulatory system.¹³⁰ It is plausible that tDCS, which influences activity in the anterior cingulate cortex,⁶⁹ could enhance deficient endogenous pain modulatory activity in these patients through its effects on the ACC and brainstem regions involved in DNIC.

Increase in Sensory Thresholds

A secondary aim of this investigation was to determine whether active tDCS alters the threshold for sensory perception as compared with sham tDCS. Using VFH monofilaments to measure sensory threshold, we found that active tDCS of the primary motor cortex does significantly increase the threshold for perceiving mechanical pressure sensation when compared to sham tDCS. This finding is consistent with previous literature. For instance, Boggio and

colleagues demonstrated in healthy subjects that anodal tDCS of the motor cortex increased thresholds for sensing a peripheral electrical stimulus by 6.5%.¹³¹ They also found thresholds for perceiving this stimulus as painful to increase by 8.3%. As expected, they did not observe significant alterations in sensory thresholds with stimulation of the dorsolateral prefrontal cortex, occipital cortex, or sham stimulation. Whereas Boggio and colleagues utilized peripheral electrical stimuli, we used mechanical stimuli (VFH). Therefore, our experiment contributes the finding that motor cortex stimulation using tDCS can increase thresholds for perceiving mechanical pressure sensation as well.

The effects of anodal tDCS on sensory thresholds are thought to be mediated through similar mechanisms as its effects on pain thresholds. That is, motor cortex stimulation likely modulates sensory and pain perception via corticothalamic modulation of epicritic (touch) and nociceptive neural activity in the ventral posterolateral and ventral posteromedial nuclei of the thalamus, respectively.¹³¹ Alternatively, it is possible that direct stimulation of the nearby somatosensory cortex during administration of motor cortex stimulation may mediate the observed increases in sensory thresholds.^{131,132}

Of note, in designing our study, we chose not to examine the effects of DNIC on sensory threshold. Subjects are required to have their contralateral hand in painful, ice-cold water during induction of DNIC. Von Frey hair sensory testing is relatively time-consuming to administer. For practical and ethical reasons, we therefore decided to examine only our primary outcome of pain threshold during DNIC-induction and not require subjects to undergo additional VFH testing.

Review of Adverse and Cognitive Effects

No significant adverse effects were reported in this study and the side effects that were reported, such as brief tingling sensation, were consistent with previous studies.^{68,124} To monitor for subtle adverse effects of tDCS on cognitive functioning we administered several cognitive assessments. As expected, we did not observe significant differences in cognitive test performance after active tDCS compared to sham conditions. While some previous studies have observed improved performance on cognitive assessments following administration of tDCS, these improvements usually only take place after multiple stimulation sessions and involve stimulation of other cortical regions such as the prefrontal cortex and posterior temporo-parietal junction.¹³³⁻¹³⁵

Insights from Magnetic Resonance Spectroscopy

Using the occipital cortex as our control, we explored whether baseline metabolite concentrations in the motor cortex, thalamus, and anterior cingulate cortex significantly correlate with subject responses to pain modulatory interventions. We observed that pain threshold increases following active tDCS were positively correlated with baseline total-NAA in the anterior cingulate cortex and negatively correlated with baseline glutamine levels in the thalamus. These findings are consistent with previous understandings of the role of these metabolites in brain function as well as related experimental findings.

NAA has been viewed as a marker of neural density, viability, and overall function, with decreased levels often suggesting impaired neural functioning.¹⁰⁸⁻¹¹⁰ A 2002 study comparing brain metabolite concentrations in healthy subjects to those in chronic pain patients following spinal cord injury found that chronic pain patients had lower concentrations of NAA in the thalamus.¹³⁶ Low thalamic NAA levels have been observed as well in patients with chronic

neuropathic pain,¹¹² trigeminal neuralgia,¹¹³ and migraine.¹¹⁴ Furthermore, chronic pain patients have been found to have decreased concentrations of NAA in the prefrontal cortex and anterior cingulate cortex.^{137,138} More recently, researchers have demonstrated that chronic visceral pain improvement is associated with increases in NAA levels following TMS treatment.¹¹⁷ Thus, decreased NAA in pain-related regions appears to be associated with chronic pain states and to increase with pain improvement. Our observation that higher NAA is associated with greater pain threshold increases after tDCS might be explained as follows: better baseline neural functioning is more conducive to tDCS having its neuromodulatory effects. As discussed in the limitations section below, however, we cannot firmly conclude any such causal relationship from the observed correlation alone. Our observation also raises the question of whether chronic pain patients (with low baseline NAA levels) might experience relatively diminished pain modulatory effects following tDCS compared to those seen in our healthy subjects (who likely have higher baseline NAA). Given the exploratory nature of these findings, it would be too early to answer this question firmly.

Glutamine is a precursor to glutamate, which is a major excitatory neurotransmitter known to be involved in pain neurophysiology. Levels of glutamate and glutamine, which are often grouped together, can be influenced by non-invasive brain stimulation.¹³⁹ Pain relief in chronic pain patients has been linked to decreasing glutamate levels in the insular cortex.¹¹⁵ A recent TMS study found that decreased baseline levels of glutamate were associated with greater chronic pain improvement following non-invasive brain stimulation.¹¹⁷ Our findings are consistent with this previous literature and go beyond, demonstrating that lower levels of glutamine-glutamate at baseline are associated with greater pain neuromodulatory effects of tDCS, albeit in healthy subjects.

Our combined observations from this exploratory analysis suggest that MRS assessment of brain metabolite levels may potentially be a useful tool for predicting response to pain modulatory interventions. We showed that, in healthy subjects, lower baseline concentrations of thalamic glutamine are associated with higher pain threshold increases following tDCS and that higher baseline concentrations of NAA in the anterior cingulate cortex are associated with higher pain threshold increases following tDCS.

Limitations

There are several limitations to this investigation that should be addressed. Though we did not observe a significant difference between the effects of tDCS and DNIC alone, it is important to highlight that manipulation of dosage could substantially alter pain responses. tDCS, for example, could potentially be administered with varying duration and current density, and DNIC could have been induced with varying durations of immersion and water temperatures.¹⁴⁰ It would therefore be incorrect to conclude from our study that tDCS and DNIC necessarily have similar magnitudes of effect under any conditions. As the mechanisms underlying these modulatory approaches continue to be examined, the dosages of each should be adjusted to explore optimal combinations and parameters for increasing pain thresholds.

Our study design called for the DNIC paradigm to be administered both before and after tDCS application. This sequence allowed us to test pain thresholds (1) at baseline, (2) during DNIC alone, (3) after active or sham tDCS, and (4) after active/sham tDCS during DNIC (combined condition). This design assumes that the initial DNIC-induction has no carry-over effects when assessing thresholds immediately following tDCS application (during #3 above). We believe this is a fair assumption. It is supported by the brief time length of the cold-water immersion (1-minute) and the fact that we had at least 15 minutes of cognitive assessments and

20 minutes of tDCS stimulation before the post-tDCS pain threshold assessments took place. In contrast, when assessing combined tDCS and DNIC (#4 above) we assumed that there *was* a carry over effect from tDCS during DNIC administration, allowing us to consider this a combined tDCS and DNIC condition. This assumption is supported by previous studies showing that brief sessions of tDCS (up to 13 minutes) can have effects lasting up to 90 minutes⁶⁵ and the fact that the DNIC pain threshold assessments were performed within minutes after tDCS application.

We found that active tDCS combined with DNIC significantly increased pain thresholds more than DNIC alone, suggesting an additive or synergistic effect. While there also appeared to be a trend in which active tDCS combined with DNIC increased thresholds more than active tDCS alone, this did not reach the level of significance. It is unclear how this should be interpreted. A strict interpretation would suggest that combined tDCS and DNIC provides significantly enhanced neuromodulatory effects on pain compared to DNIC alone, but not compared to tDCS alone. It seems possible that with a greater sample size and higher-powered study, we might find the observed trend to reach significance and see combined tDCS and DNIC increase thresholds significantly more than tDCS alone. At this point, however, that is speculative. Importantly, we cannot conclude based on the data presented here whether the combined effects of tDCS and DNIC are additive or synergistic (i.e., equal to the sum of the effects of each administered alone [additive] vs. greater than the sum of the effects of each administered alone [synergistic]). While they appear additive, higher powered studies would be necessary to confirm this.

With regard to adverse effects, it is reassuring that we did not observe any significant adverse effects. We also did not observe any instances of acute mood changes, trouble concentrating, and neck pain. One caveat, however, is that the adverse effects questionnaire was

administered immediately following stimulation. In retrospect, it may have been beneficial to include a follow-up call to subjects the day after stimulation as well to ensure no adverse effects took place several hours after stimulation. While previous studies have not found this to occur, this would be a simple step to ensure no adverse effects are missed.^{68,124}

As a point of clarification, I refer to DNIC as “endogenous” pain modulation and tDCS as “exogenous,” because DNIC is part of an endogenous regulatory system that naturally responds to modulate pain whereas tDCS requires an “exogenous” tool to modulate pain. To be clear, however, when the motor cortex is stimulated with anodal tDCS, the downstream effects likely do involve activation of endogenous pain regulatory processes. As discussed above, for instance, it is believed tDCS influences the periaqueductal gray matter, which triggers descending inhibitory signals down the spinal cord. Thus, even “exogenous” pain interventions like tDCS will ultimately involve many endogenous processes in modulating pain.^{91,92}

It is important to emphasize that this study was conducted in healthy human subjects using acute, experimentally-induced pain and its conclusions can therefore not be generalized to chronic pain patients. It is very possible that our experimental paradigm would not produce the same results in chronic pain patients. As discussed earlier, DNIC is found to be impaired in some chronic pain populations.^{37,130} Our DNIC-induction paradigm might therefore demonstrate varying effects in these populations. Baseline brain metabolite concentrations also vary between healthy humans and chronic pain patients, with chronic pain patients having lower levels of metabolites such as NAA in certain brain regions.¹³⁶ Given these differing baseline neurophysiological states, chronic pain patients may react differently to tDCS than healthy humans. At the same time, this study provides valuable information about the effects of DNIC and tDCS in modulating pain under normal physiological conditions and, importantly, provides a point of comparison for future studies in chronic pain populations (discussed below).

With respect to our MRS findings, it is important to mention that given our small sample size and post-hoc approach, our findings must be interpreted with caution. In these post hoc exploratory analyses, there was no power to correct for multiple comparisons, a limitation of which the reader should be aware due to the higher false positive rate. Several associations between metabolite concentrations and pain responses were observed to take place in both the occipital cortex and our regions of interest. Since the occipital cortex was our control region for the analysis, we deemed these associations insignificant. In contrast, the significant associations with NAA and glutamine discussed above were observed only in our regions of interest and not the control occipital region. While we deemed these significant, these associations must still be interpreted with caution, due to the post hoc nature of these analyses and lack of Bonferroni correction.

Finally, it is necessary to highlight that these correlations between baseline metabolite levels and pain modulation responses do *not* imply causation. Even if we could confirm that various brain metabolite levels are strongly associated with response to pain modulatory interventions, these metabolites would not necessarily have to be in the mechanistic pathway mediating neuromodulatory response. For instance, a separate biological variable may both cause high levels of NAA and facilitate neuromodulatory response, allowing NAA to serve as a biomarker or predictor of response, while still not being on the mechanistic pathway mediating neuromodulatory response.

Future Directions

The current study suggests many potential directions for future research. We should continue testing new technologies, techniques, and parameters as we attempt to identify even more effective pain modulatory approaches. For example, the present paper explores the effects

of *anodal* tDCS on pain thresholds. This follows from a vast literature demonstrating anodal stimulation to be efficacious in healthy and chronic pain populations.^{94,96,105,141,142} *Cathodal* tDCS also appears to exert significant effects in modulating experimental pain.¹⁴³⁻¹⁴⁵ Future studies should therefore explore the pain modulatory effects of cathodal tDCS and other stimulation techniques such as alternating current tDCS.⁵⁷ We should aim to maximize pain modulation by varying other tDCS parameters as well, such as electrode size, current intensity, duration, stimulation site, and number of stimulation sessions.⁹² For instance, 4x1 ring high-definition tDCS (HD-tDCS) is a promising new tool that uses an array of smaller electrodes to allow for greater focusing of current on stimulated regions.¹⁴⁶ We should explore stimulation of other cerebral cortical regions as well, such as the dorsolateral prefrontal cortex, since they may influence pain through different mechanisms.^{147,148} Parameters for inducing DNIC can be modified as well by using different noxious stimuli (cold, electrical, heat, mechanical) for varying durations and number of repetitions.

Eventually, the most effective techniques identified should be tested in clinical trials with chronic pain patients so their therapeutic value can be evaluated. The present study, after all, was performed in healthy human subjects and its results cannot be generalized to chronic pain patients, who may react very differently to pain modulation techniques. We should explore the possibility of enhancing existing pain therapies, whether pharmacologic or otherwise, through combinations with various neuromodulation techniques.¹⁰⁴⁻¹⁰⁶ We might also attempt combining various neuromodulatory tools such as tDCS and TMS. As we plan for these clinical trials, we need to be cautious in choosing our inclusion and exclusion criteria. While this thesis often refers to chronic pain in general terms, chronic pain is in fact a highly heterogeneous condition and its pathophysiology can vary greatly between types. Thus we need to take care to pick well-defined patient populations for trials and prevent ourselves from overgeneralizing results.

We should also aim to identify and understand pain therapies that employ DNIC analgesic mechanisms. For instance, some have theorized that non-pharmacologic pain therapies such as acupuncture and transcutaneous electrical nerve stimulation may contribute to analgesia, in part, through activation of DNIC.^{7,42-44} Endogenous pain modulation may also provide a mechanistic basis for placebo-induced analgesia.⁴¹ A better understanding of the neurophysiology of DNIC and its roles in existing pain therapies may reveal new paradigms for pain treatment. Furthermore, the manner in which attentional factors influence DNIC's effect on pain when combined with tDCS and other therapies ought to be explored.¹⁴⁹

Much remains to be understood regarding the neurophysiology underlying the effects of tDCS. As discussed above, tDCS exerts its effects by directly and indirectly modulating activity in a wide array of superficial and deep neural structures. Future research in healthy and patient populations should utilize brain imaging to help elucidate the mechanisms of tDCS's effects in more detail. For instance, we might scan subjects using functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), and electroencephalography (EEG) before, during, and following the administration of tDCS. This combination would provide high spatial resolution (MRS), data on neurochemical changes and glucose metabolism (PET, MRS), and information on system wide, oscillatory changes (EEG). Such imaging modalities applied to awake animal models might prove extremely valuable in further probing the neurophysiological effects of tDCS.

The results from the exploratory MRS analyses in the present study suggest that baseline metabolite levels may be associated with response to neuromodulatory interventions. Future work should more rigorously test these associations both in healthy and patient populations. Our study design did not include a post-stimulation MRS session, because subjects only underwent a single short stimulation session. Follow-up investigations should involve multiple sessions as

well as pre- and post-stimulation MRS scans to evaluate neurochemical changes involved in pain modulation.

Furthermore, future research should explore the utility of MRS as a potential diagnostic and prognostic tool in the area of chronic pain. Harris and Clauw proposed that MRS might be used to develop “personalized” analgesic therapy for chronic pain patients.¹¹¹ This proposal developed from the observation that there are many types of chronic pain and no single medication that helps all patients. They proposed pre-treatment scanning of chronic pain patients with MRS to assess dysfunctional levels of brain metabolites such as glutamate and GABA, which could then be used to “personalize” treatment. For instance, patients with elevated levels of glutamate (the excitatory neurotransmitter involved in pain) might be treated with a drug that targets glutamatergic neurotransmission, such as pregabalin. Similarly, those patients with low levels of GABA (the neurotransmitter that inhibits pain pathways) might receive GABA-ergic medications, such as beta-hydroxybutyrate. Patients with both findings, could be treated with dual therapy.¹¹¹ This exciting proposal could potentially lead to a paradigm shift in pain therapeutics, transitioning us away from the current trial-and-error standard and toward a more calculated, mechanistically focused approach.

Finally, from a health policy perspective, there are important measures that can be taken to advance pain research generally. As mentioned in the opening of this thesis, chronic pain constitutes a major burden of disease in society, with 1.5 billion people worldwide estimated to suffer from various forms.¹ While more individuals in the US are affected by chronic pain than cancer, diabetes, and heart disease combined, pain research is currently granted less than 1% of the budget of the National Institutes of Health (NIH).^{1,2} Over the last half-century, fewer than 10 pharmaceutical drugs with new mechanisms of action have been introduced. Only one medication now being used clinically has been designed based on targeted mechanisms of action

(i.e., triptans for migraine treatment).² Pain represents a major public health challenge that demands prompt attention. The federal government and NIH ought to commit more funding to support research that aims to better understand the neural mechanisms underlying physiologic and pathologic pain as well as to develop novel pharmacologic, neuromodulatory, and other treatments for acute and chronic pain. More effective integration of pain research at the National Institutes of Health and in academic centers nationally would help to further these goals.²

Summary and Conclusions

This thesis explored whether combining exogenous and endogenous pain modulation approaches (anodal tDCS of M1 and a DNIC-induction paradigm, respectively), synergistically increases the threshold at which pain is perceived. We conducted a double-blinded, randomized, placebo-controlled trial with a crossover design in 15 healthy human subjects. We found that, when administered alone, both tDCS and DNIC significantly increased the thresholds at which subjects perceive pain. When combined, these modulatory approaches appeared to have additive effects. tDCS also significantly increased the threshold for perceiving sensation when compared to sham conditions. Finally, baseline concentrations of brain metabolites were significantly associated with the effects of tDCS on pain thresholds. Increases in pain threshold after tDCS were positively correlated with baseline levels of NAA in the anterior cingulate cortex and negatively correlated with baseline levels of glutamine in the thalamus. Future research should explore whether existing pain therapies may be improved with noninvasive brain stimulation and activation of endogenous pain regulatory systems such as DNIC. We should work to enhance pain modulation using alternative parameters for tDCS and DNIC-induction and to understand the neurophysiology underlying their effects. Finally, we ought to examine whether brain metabolite concentrations can be utilized to predict clinical response to therapeutic interventions.

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Table 1. Varying Parameters of tDCS

Parameter	Standard Range	Effect
Electrode Size	20 cm ² – 35 cm ²	Smaller electrode size results in greater final cortical current density, but also greater shunting to the scalp. Unipolar stimulation can be achieved through a small electrode by enlarging the area of the other electrode.
Current Intensity	1.0 mA – 2.0 mA	A current intensity of 0.6 mA is necessary to observe after-effects. Larger current intensity results in greater amplitude of effect (as measured by MEPs) and longer-lasting effects.
Current Density on Scalp Surface	24 µA/cm ² – 29 µA/cm ²	Larger current densities result in stronger effects of tDCS. Lower current densities (less than 24 µA/cm ²) for a few minutes do not induce any significant effects. (This is the ratio of current intensity and electrode size).
Stimulation Duration	5 min – 30 min	Longer duration results in longer-lasting effects. Whereas 5 to 7 minutes of tDCS results in after-effects lasting for no longer than 5 minutes, tDCS from 9 to 13 minutes results in after-effects lasting from 30 to 90 minutes, respectively.
Stimulation Polarity	Anodal or Cathodal (applied to cortical region of interest)	Effect depends strictly on the orientation of axons and dendrites in the induced electrical field. Generally, anodal tDCS increases the excitability of the underlying cortex by depolarizing neuronal membranes to subthreshold levels, while cathodal tDCS applied over the same area decreases it by hyperpolarizing neuronal membranes.
Stimulation Site	M1, V1, Somatosensory Cortex, Dorsolateral Prefrontal Cortex	Site-specific and differential effects on a gamut of cognitive, behavioral, psychosomatic, and electrophysiological tests. While the polarizing effects of tDCS are generally confined to the areas under the electrodes, the functional effects appear to perpetuate beyond the immediate site of stimulation. Anodal tDCS of the premotor cortex, for instance, increases excitability of the ipsilateral motor cortex and inhibition of contralateral motor areas.

Note: (Table excerpted with permission from Reidler et al. (2011)¹⁵⁰)

Table 2. Side effects of tDCS Administration

	Active tDCS	Sham tDCS
Tingling	11 (73)	10 (67)
Skin Redness	7 (47)	8 (53)
Sleepiness	5 (33)	6 (40)
Itching	2 (13)	1 (7)
Scalp Pain	1 (7)	2 (13)
Scalp Burning	1 (7)	0 (0)
Headache	1 (7)	1 (7)
Pins and Needles	1 (7)	0 (0)
Neck Pain	0 (0)	0 (0)
Trouble Concentrating	0 (0)	0 (0)
Acute Mood Change	0 (0)	0 (0)

Note: Number of subjects and percentage (in parentheses) reporting the side effect. (Table excerpted with permission from Reidler et al. (2012).¹⁵¹)



Figure 1. Transcranial Magnetic Stimulation. A copper wire coil encased in plastic is placed on the subject's scalp overlying the cortical region to be stimulated. As current is sent through the coil, a 2 Tesla magnetic field is generated in a perpendicular plane to the coil. This rapidly generated magnetic field penetrates the subject's scalp and skull, inducing electrical current in the underlying cortical regions strong enough to depolarize cellular membranes and induce neuronal activity. (Figure excerpted with permission from Reidler et al. (2011)¹⁵⁰).



Figure 2. Transcranial Direct Current Stimulation. Two rubber electrodes are placed in saline-soaked sponges and then applied to the scalp. The electrodes are attached by wire to a battery-powered direct current generator. The tDCS operator sets the device's internal resistance with a dial to reach a target current ranging from 0.5 mA to 2.0 mA. Current travels through the scalp and skull to the underlying cortex, inducing real-time neurophysiological effects. (Figure excerpted with permission from Reidler et al. (2011).¹⁵⁰ Redacted additional image of transcranial direct current stimulator excerpted from neuroConn.¹⁵²)

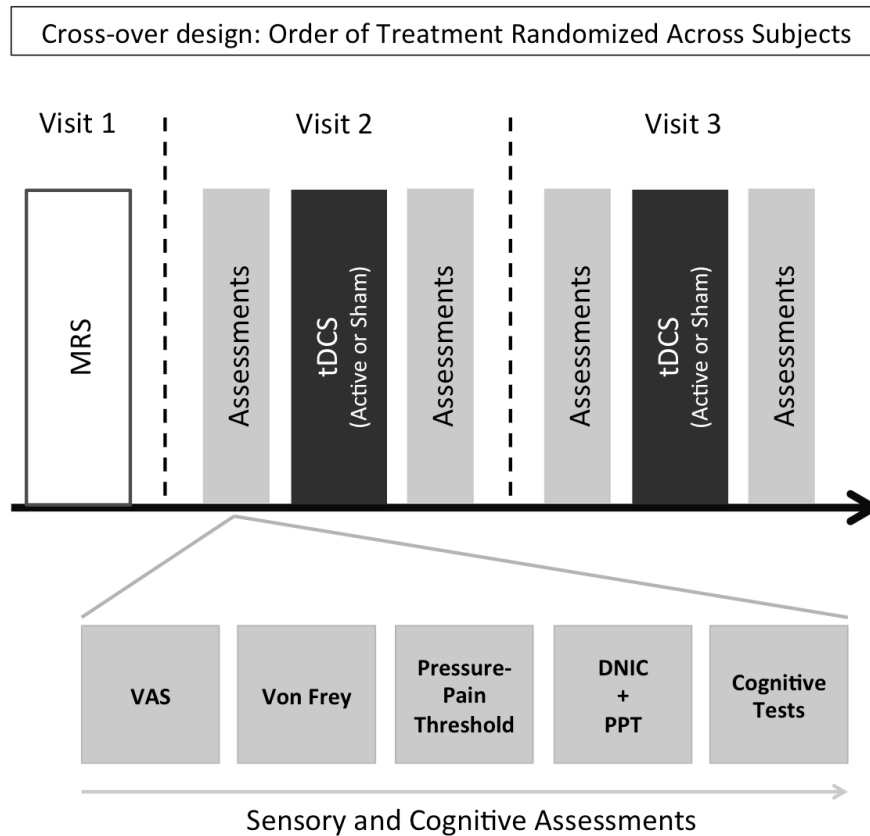


Figure 3. Experimental Design for Evaluating the Effects of tDCS and DNIC on Pain Thresholds. The study design involved three visits. During Visit 1, subjects underwent magnetic resonance spectroscopy (MRS) to measure baseline metabolite concentrations in pain-related regions of interest. During Visit 2, subjects were randomized to receive active or sham anodal tDCS. This was preceded and followed by a series of assessments, which included the Visual Analog Scales (VAS) for anxiety, Von Frey Hair sensory threshold test, Pressure-Pain Threshold (PPT) measurements performed using an algometer on the right thenar region, PPT following the first 30 seconds of left hand cold-water immersion (DNIC paradigm), and cognitive assessments. During Visit 3, subjects underwent identical procedures as in Visit 2, with the only change being the active vs. sham stimulation conditions. This design allowed us to evaluate the effects of active tDCS, sham tDCS, DNIC, and combined conditions in all subjects (cross-over design). (Figure excerpted with permission from Reidler et al. (2012).¹⁵¹)

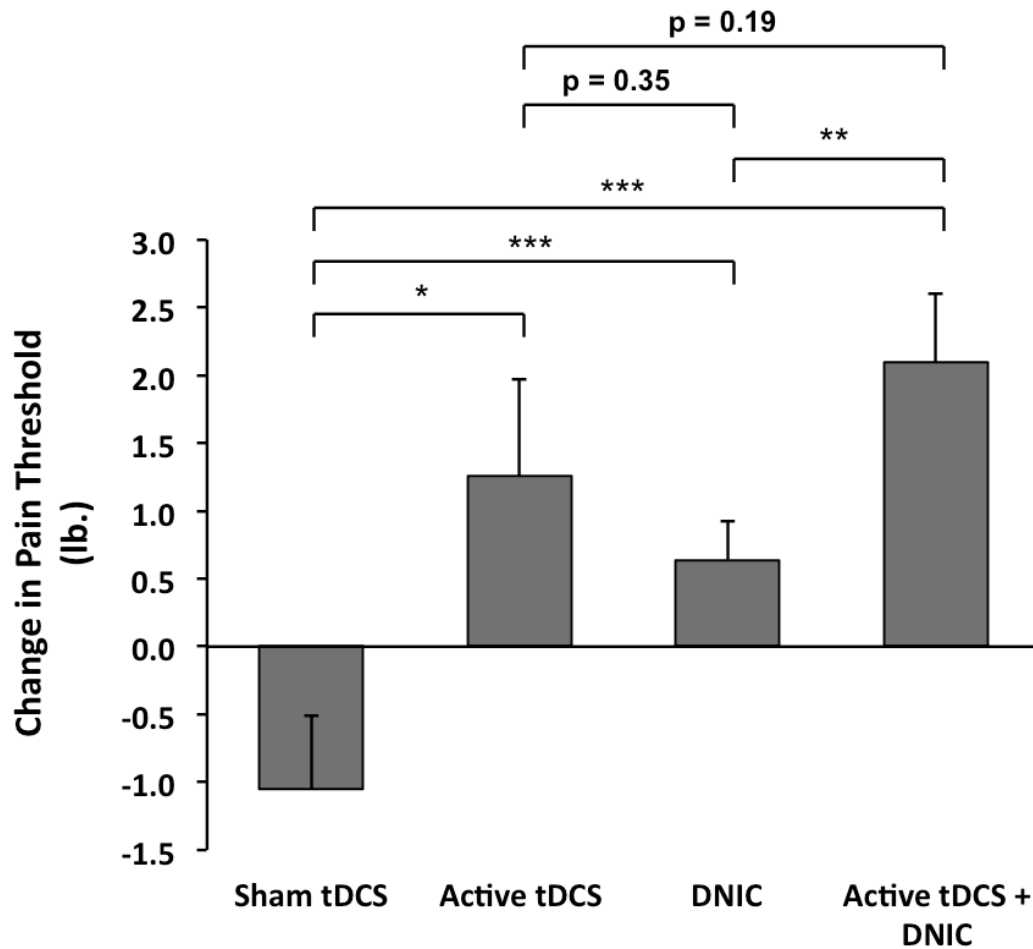


Figure 4. Motor Cortex Stimulation and DNIC Increase Pain Thresholds. We compared subjects' pain thresholds at baseline to their thresholds following administration of sham tDCS, active tDCS, DNIC, and combined active tDCS and DNIC. When compared to sham conditions, significant increases in pain thresholds were observed following active tDCS, DNIC, and combined conditions. Change in pain thresholds after active tDCS alone was not significantly different from change observed after DNIC alone. Combined active tDCS and DNIC increased pain thresholds more than either method alone, and this reached significant levels when compared to DNIC conditions. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$ (paired t -test, 2-tailed). (Figure excerpted with permission from Reidler et al. (2012).¹⁵¹)

Appendix A. tDCS Screening Questionnaire

Subject Initials: _____ IRB Protocol # _____

Date: ____/____/____ Time of Study _____

Investigator Name: _____

Short Study Title: _____

TMS/ TDCS Screening Questionnaire

Have you ever:

Had TMS/ tDCS before? _____ Yes _____ No

Had an adverse reaction to TMS/tDCS? _____ Yes _____ No

Had a seizure? _____ Yes _____ No

Had an unexplained loss of consciousness? _____ Yes _____ No

Had a stroke? _____ Yes _____ No

Had a serious head injury? _____ Yes _____ No

Had a surgery to your head? _____ Yes _____ No

Had any brain related, neurological illnesses? _____ Yes _____ No

Had any illness that may have caused brain injury? _____ Yes _____ No

Do you suffer from frequent or severe headaches? _____ Yes _____ No

Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips,
or fragments from welding? _____ Yes _____ No

Do you have any implanted medical devices such as cardiac pacemakers or medical pumps? ☐ Yes ☐ No

Are you taking any medications? ☐ Yes ☐ No

Are you pregnant, or are you sexually active and not sure whether you might be pregnant? ☐ Yes ☐ No

Does anyone in your family have epilepsy? ☐ Yes ☐ No

Do you need any further explanations on TMS/ tDCS or its associated risks? ☐ Yes ☐ No

For any yes responses please provide detailed information:

Subject Signature

Date: ____/____/____

Investigator Signature

Date: ____/____/____

Appendix B. Visual Analog Scale for Anxiety

4825317819 <input type="text"/>	Principal Investigator/Program Director (Last, First): Fregni, Felipe
Subject Initials <input type="text"/>	Subject ID Number <input type="text"/>

Appendix D - VAS Anxiety

Date / /

Week Day

0 .5 1 .5 2 .5 3 .5 4 .5 5 .5 6 .5 7 .5 8 .5 9 .5 10

Not Anxious/
Completely Calm Very
Anxious

Instructions:

Please fill out anxiety scale at the same time each day. Try to make it a time of day when your anxiety tends to be at its worst.

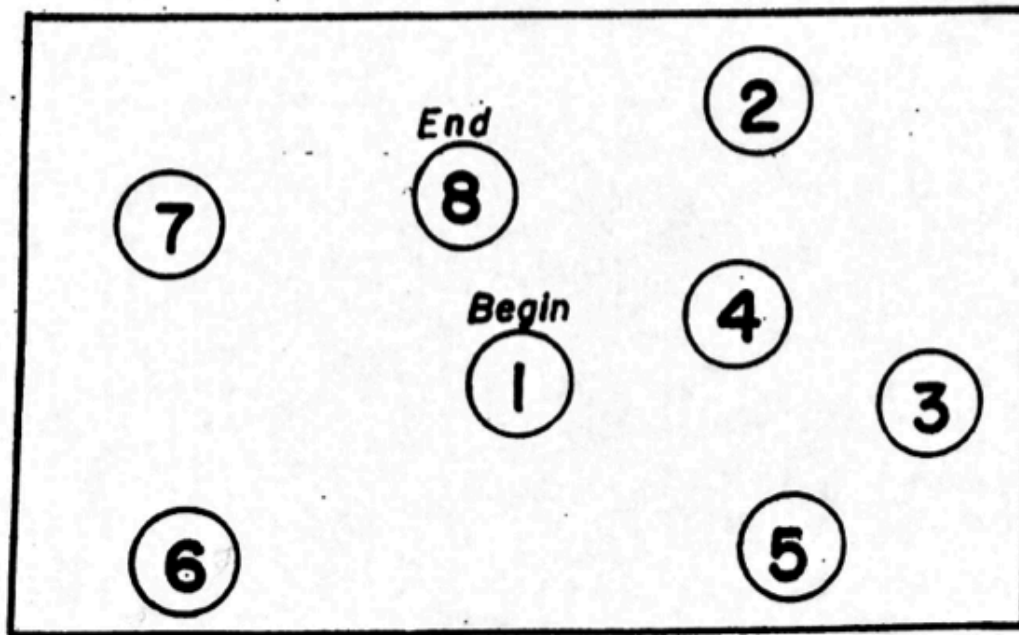
Please color-in the circle (●) that best describes your anxiety at that moment in time.

Please bring your completed set of sheets for each weekly visit in exchange for the next week of sheets.

TRAIL MAKING

Part A

SAMPLE

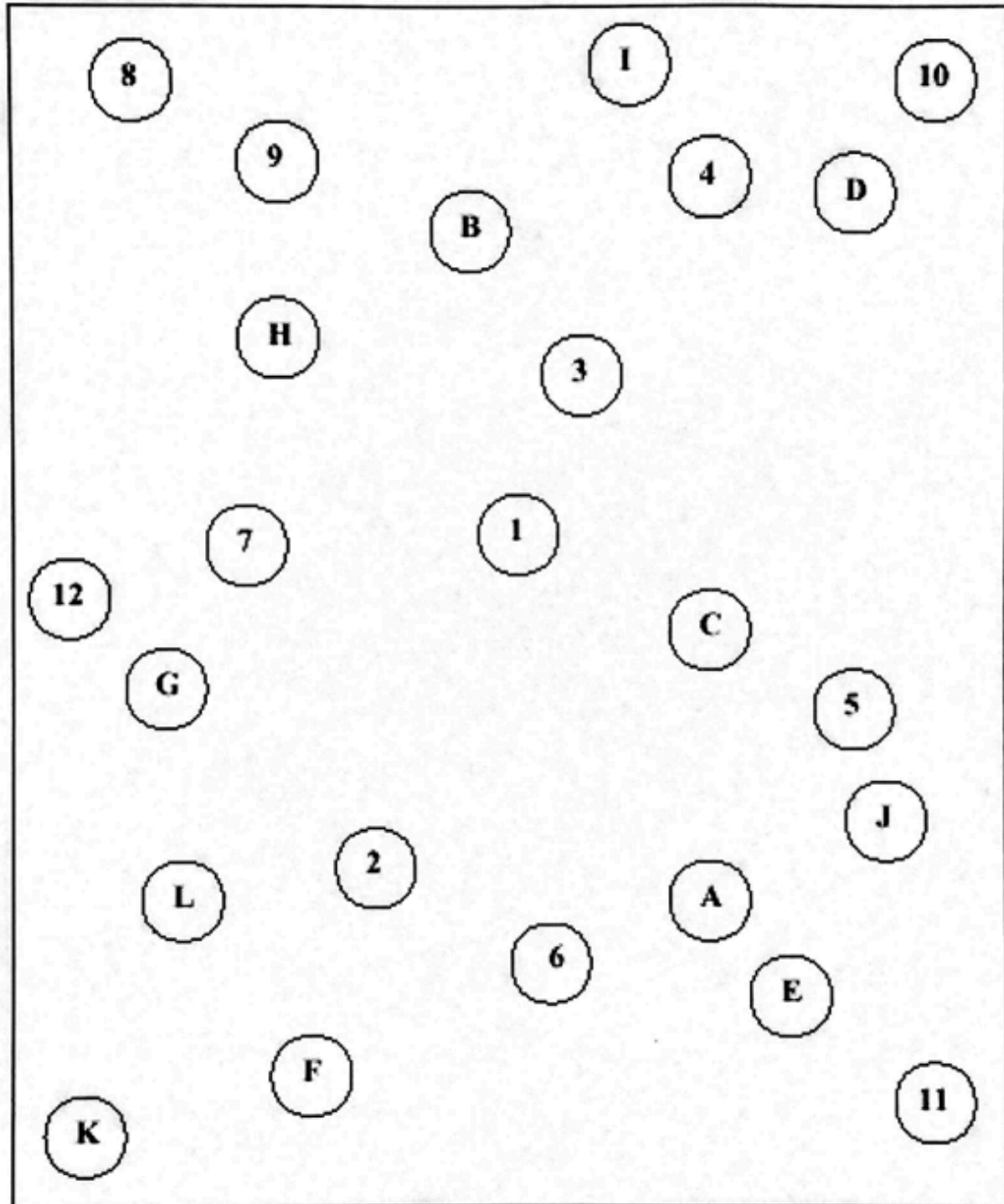




Trail Making Test Part B

Patient's Name: _____

Date: _____



Appendix D. tDCS Side Effects Questionnaire

tDCS Side Effects Questionnaire – Session _____

Patient Initials: _____

Date: _____

<i>Do you experience any of the following symptoms or side effects?</i>	<i>Enter a value (1-4) in the space below. 1-Absent 2-Mild 3-Moderate 4-Severe</i>	<i>If present: Is this related to tDCS? 1-None 2-Remote 3-Possible 4-Probable 5-Definite</i>	<i>Notes</i>
Headache			
Neck Pain			
Scalp Pain			
Scalp Burns			
Tingling			
Skin Redness			
Sleepiness			
Trouble Concentrating			
Acute Mood Change			
Other (specify):			

Appendix E. tDCS Blinding Questionnaire

Validation of Blinding

PI: Fregni, Felipe

Stimulation Confidence Rating

Answer the questions to the best of your ability:

Did you receive:

☐ Placebo Stimulation

☐ Active Stimulation

Rate how confident you feel in your answer (please check one):

☐ 1 Not confident at all

☐ 2

☐ 3

☐ 4

☐ 5 Completely confident